UNIVERSITI TEKNOLOGI MARA

DETERMINATION OF THE EFFECTS OF *PIPER BETLE* LEAF ETHANOLIC EXTRACT ON MYELOPEROXIDASE ACTIVITY AND *mpo* GENE EXPRESSION IN NEUTROPHILS IN RESPONSE TO *STAPHYLOCOCCUS AUREUS*

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of the requirement for the degree of
Master of Sciences

Faculty of Health Sciences

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a panel of Examiners has met on 10th March 2015 to conduct the final examination of Yalda Modirrahmati on her Master of Science thesis entitled "Determination of the effects of *Piper betle* leaf ethanolic extract on myeloperoxidase activity and *mpo* gene expression in neutrophils in response to *Staphylococcus aureus*" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The panel of examiners recommends that the student be awarded the relevant degree. The panel of examiners was as follows:

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the instruction of Universiti Teknologi MARA. This manuscript is original, has not been published before and is not currently being considered for publication elsewhere, academic institution or non-academic institution for any other degree or qualification.

I hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Piper betle leaves are consumed in traditional medicine in many Asian countries including Malaysia and it has antimicrobial and antioxidant activities. *Staphylococcus aureus* is a troublesome pathogen due to its ability to cause infections that withstand multiple antibiotics. Myeloperoxidase (MPO) is a heme enzyme gene encoding myeloperoxidase (*mpo*) with a molecular mass of 150 kDa consisting of two alpha-chains 60 kDa and two beta-chains 15 kDa. It is abundantly expressed in neutrophils (PMNs) and generates hypochlorous acid (HOCl) during respiratory burst which is utilized to kill bacteria. The effects of predetermined *P. betle* ethanolic leaf extract MIC (5 mg/ml) on MPO in human neutrophils that were challenged with *S. aureus* (ATCC 25923) were investigated. MPO expression was analyzed using SDS-PAGE and total protein concentrations in neutrophil lysates were determined using Bradford assay. SDS-PAGE analysis showed significant effects of the extract on MPO after just 1 hour treatment. Based on observed effects, the protocol was adapted for use in all proceeding assays. Myeloperoxidase activity was determined using a kit assay (MPO activity colorimetric detection kit, USA) and effect on neutrophil killing activity against *S. aureus* was tested. Further, *mpo* expression analyses were performed using quantitative real-time PCR and validated using the Livak calculation method. SDS-PAGE analyses showed that *S. aureus* represses expression of both the alpha and beta chains of MPO. Notably, expression of the beta chain was affected more drastically. A corresponding 19.23% reduction in protein concentrations observed in treated PMNs with *S. aureus* as determined by the Bradford assay. *P. betle* extract significantly alleviated the repressor effect by *S. aureus* whereby band densities of both subunits were increased. Corresponding increases in protein concentrations at 19.23% and 13.44% were seen in PMNs treated with both extract and *S. aureus* together and with *P. betle* extract only respectively. Myeloperoxidase activity analysis showed 18.33 mU/ml increased in presence of *P. betle* extract compared to untreated PMNs 3.33 mU/ml while PMNs treated with *S. aureus* showed no MPO activity. Significantly, increase of 22.5 mU/ml was observed in treated PMNs with *P. betle* extract even in presence of *S. aureus* which corresponds to increased protein profile observed in both SDS-PAGE analysis and Bradford assay. The neutrophil killing assay revealed significant effect of *P. betle* ethanolic extract on neutrophil killing activity. Neutrophils treated with *S. aureus* showed 79.24% survival whereas addition of *P. betle* extract reduced survival to only 7.14% even in the presence of the organism. *mpo* expression analysis by real-time PCR revealed increased expression 2.29 fold after treatment of neutrophils by *P. betle* extract in the presence of *S. aureus*. A similar repressor effect was observed in PMNs treated with *S. aureus* which revealed only 0.053 fold gene expression compared to untreated neutrophils. Overall data obtained from this study confirms that *S. aureus* showed downregulates *mpo* expression resulting in reduced protein expression, enzyme activity and neutrophil killing ability. *P. betle* ethanolic extract overcomes these repressive effects resulting in corresponding increase in all identical parameters that were tested. This study potentially aids in discovery and development of new antimicrobials. The findings in this study elucidated mechanisms by which the ethanolic extract of *P. betle* significantly affects clearance of *S. aureus in vitro.* Future work involving *in vivo* studies would be beneficial to ascertain if the observable effects can be replicated. Animal model studies would reveal if the extract can be potentially developed to enhance the innate immune system, particularly involving killing potential of neutrophils and clearance of *S. aureus.*

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